

Micropropagation of *Elaeis guineensis* Jacq. 'Dura': Comparison of three basal media for efficient regeneration

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Three basal plant tissue culture media, namely, N₆, MS, and modified Y₃, were compared to optimize micropropagation protocol for *E. guineensis*. Full strength media were used separately to regenerate plantlets directly using immature zygotic embryos (IZEs), and through somatic embryogenesis of calli obtained from IZEs. The plantlets regenerated by direct regeneration on three media were examined for shoot length and rooting percentage. For the induction of callus, somatic embryogenesis, and rooting modified Y₃ medium was the most effective. In conclusion, the results indicate that modified Y₃ medium is the most suitable for direct regeneration, callus induction and somatic embryogenesis in *E. guineensis*.

Keywords: *Elaeis guineensis*, *Elaeis oleifera*, Micropropagation, Oil palm

African oil palm, *Elaeis guineensis* Jacq. has three different forms (also called as varieties), namely, 'Pisifera', 'Dura', and 'Tenera' distinguished on the basis of shell thickness of fruits. Oil palm, *Elaeis guineensis* Jacq. 'Tenera' is a hybrid of 'Dura' (♀) and 'Pisifera' (♂), and highly preferred for the commercial cultivation because of high oil yield derived from fruit mesocarp tissue. In breeding commercial palms, 'Dura' form is an important source of germplasm^{1,2}.

In vivo vegetative propagation of oil palm is not practical because it has only one growing point. Oil palm is monoecious, cross-pollination is common and the value of parent plants is determined by the performance of the progeny produced in such crosses. Fruit bunch-yield, size of the kernel in fruits and oil content of fruits are used as criteria for selecting individual palms for breeding. Normally, *E. guineensis* Jacq. 'Dura' is propagated by seeds. However, relatively low seed-germination rate and difficulties in seedling establishment are the constraints. Therefore, an efficient protocol for the

micropropagation has a distinct advantage in accelerating the propagation of elite oil palm clones. Immature zygotic embryos (IZEs) are being used as explants in micropropagation³ and it is now possible to produce large numbers of individual somatic embryos with synchronized development^{4,5}. IZEs are also preferred as target tissue in oil palm genetic transformation studies⁶ and to study somaclonal variation⁷. However, there is no report on the comparative suitability of the different basal media. With a view to find out the most suitable medium for the micropropagation of *E. guineensis* Jacq. 'Dura', an attempt has been made in this study to compare three basal media, namely N₆⁸, MS⁹, and modified Y₃¹⁰.

Materials and Methods

Ten weeks after anthesis (WAA) fresh fruits of *E. guineensis* Jacq. 'Dura' were harvested from an open pollinated, field grown palm in the Pamol Plantation, Kluang, Johore, Malaysia. Fruits were separated from the compact fruit bunch. Persistent calyx and corolla were removed from each fruit by using surgical blade, and fruits of uniform size were selected for use. For fruit surface decontamination fruits were washed with 4% Decon 90 (Decon Laboratories Ltd., Briton) soap solution. After washing with plenty of running tap water, the fruits were immersed in 0.05% TWEEN[®] 20 (Polyoxyethylene Sorbitan Monolaurate; Amersham

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Life Science) solution for 10 min. The fruits were cleaned 4 to 5 times with plenty of distilled water after TWEEN[®] 20 treatment and were taken in the laminar air-flow cabinet. In laminar air-flow cabinet, fruits were washed in small batches (60–80 fruits) with sterile distilled water and then were immersed in 100% alcohol (Industrial grade) for 10 min. Finally, fruits were transferred on sterile tissue paper and air-dried in laminar air-flow cabinet. The fruits were cut into halves and IZEs were isolated aseptically from

the endosperm. The excised IZEs were used for direct regeneration and callus induction.

IZEs (50) were inoculated on each semisolid media (Table 1) with no plant growth regulators in one set, while in another set, media were supplemented with 2 mg l⁻¹ IBA to promote the rooting. All inoculated IZEs (in Petri plates) were incubated at 26°±2°C, under 16 hr photoperiod. After 6 weeks, germinated IZEs were shifted to 40 ml test tubes containing freshly prepared same medium, and incubated under

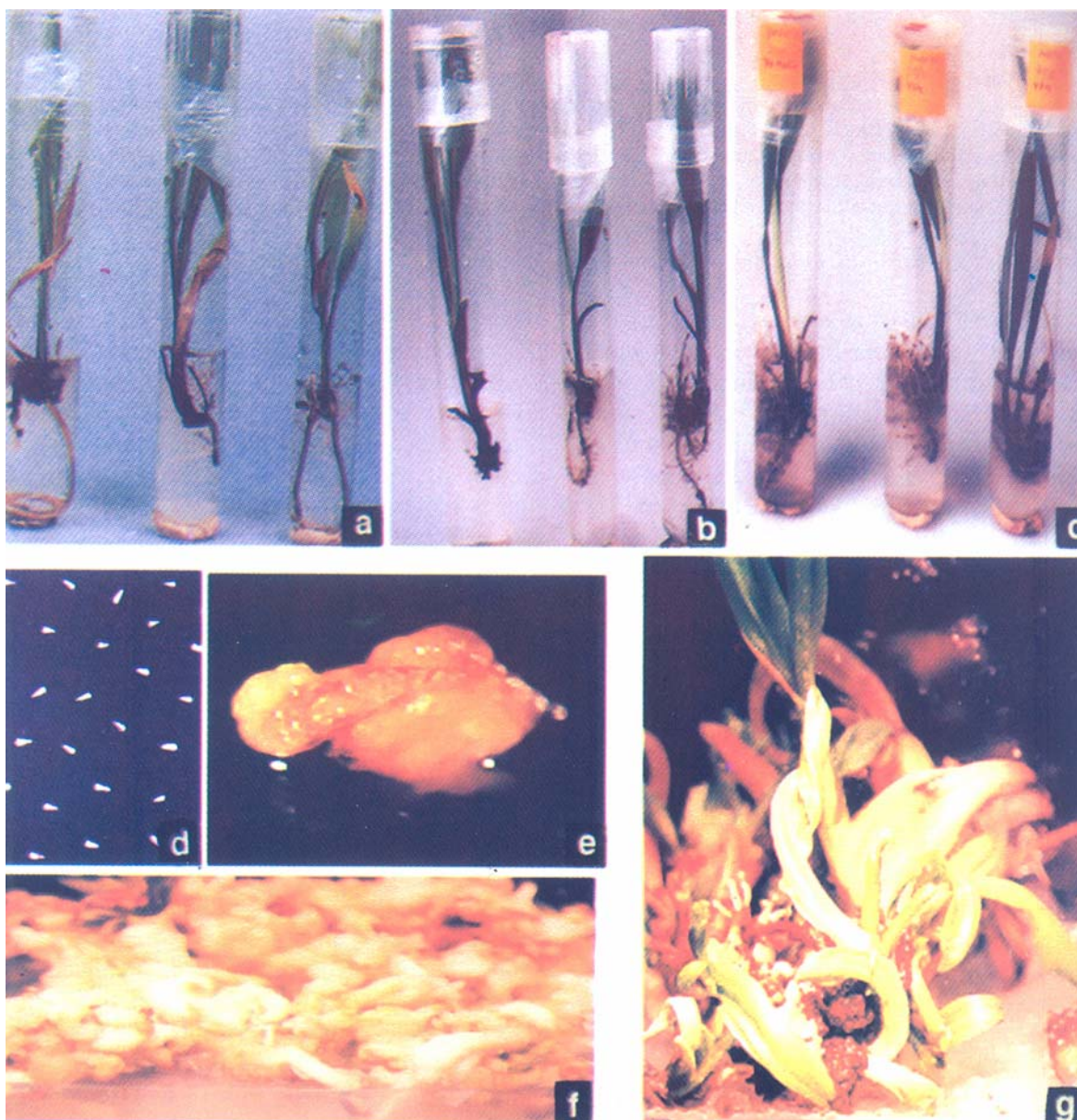


Fig. 1—Micropropagation of *E. guineensis* using immature zygotic embryos (IZEs). (a) – plantlets regenerated on N₆ medium; (b) – plantlets regenerated on MS medium; (c) – plantlets regenerated on modified Y₃ medium; 5 month-old plantlets in plate a, b and c are from three respective media that were supplemented with 2 mg l⁻¹ IBA; (d) – IZEs isolated from 10 week-old fruits of *E. guineensis*; (e) – callus formed by an IZE; (f) – somatic embryogenesis of the callus on modified Y₃ medium; and (g) – germinated somatic embryos.

the same conditions. Once in a month sub-culturing was carried out. After 5 months incubation, shoot length and rooting percentage were recorded.

For callus induction, 50 IZEs were inoculated separately on each semisolid medium [N_6 , MS, and modified Y_3], each supplemented with 2.5 mg l^{-1} 2,4-D. The cultures were incubated at $26^\circ \pm 2^\circ\text{C}$ in the dark. After 4.5 months, induced calli were shifted on the same freshly prepared media supplemented with 2.5 mg l^{-1} 2, 4-D and 2 mg l^{-1} NAA, and incubated under the same conditions. Time required for the induction of somatic embryogenesis, and percentage of the somatic embryogenesis was monitored. For the regeneration of plantlets, somatic embryos were incubated under 16 hr photoperiod at $26^\circ \pm 2^\circ\text{C}$.

Results and Discussion

Plantlets were successfully regenerated directly from IZEs on N_6 , MS, and modified Y_3 media. Five month-old plantlets, regenerated on media supplemented with 2 mg l^{-1} IBA are shown in Fig. 1 (a-c). By the end of 5 months incubation, there was no significant difference in the shoot length of plantlets regenerated on three different media both in presence and in absence of auxin (IBA). However, there was significant difference in rooting percentage, intensity of rooting, and rooting patterns. The plantlets regenerated on N_6 , MS, and modified Y_3 media showed 14, 18, and 62% rooting respectively in absence of auxin (IBA). While, regenerated plantlets showed 20, 36, and 96% rooting on N_6 , MS, and modified Y_3 media respectively, when supplemented with 2 mg l^{-1} IBA. Root polymorphism was observed (Fig. 1 a-c), and roots were classified as primary vertical and horizontal type based on root classification of oil palm given by Christophe and Rey¹¹.

The plantlets regenerated on the modified Y_3 medium, both in presence and absence of IBA showed high percentage and profuse rooting, which could be attributed to content of medium. Modified Y_3 medium contains higher concentration of KCl, which provides more Cl^- ions. The Cl^- ions are known to act like natural auxins in the root induction and their growth¹². Modified Y_3 medium is the richest source of amino acids, which might be playing a vital role in part. In addition to this, modified Y_3 medium contains 15 g l^{-1} more sucrose (carbon source) as compared to N_6 and

MS basal media (Table 1). Media rich in nutrients are known to help explants to grow well¹³.

In callus induction experiment 80, 82, and 98% IZEs turned into callus on N_6 , MS, and modified Y_3 basal media respectively. In one month, 4, and 8% calli lumps turned into embryogenic calli on MS, and modified Y_3 medium respectively. However, not a single calli lump turned into embryogenic calli on the N_6 medium.

The results indicate that modified Y_3 medium is the most suitable among the three basal media compared, for both direct regeneration and somatic embryogenesis of *E. guineensis* Jacq. 'Dura'. Data analysis by Duncan's Multiple Range Test¹⁴ (DMRT-

Table 1—Nutrient composition of the three media formulations used in this study

Chemicals	Concentration (mg l^{-1})		
	N_6	MS	Modified Y_3
Macro-nutrients			
NH_4NO_3	-	1650.00	-
NH_4Cl	-	-	535.00
KNO_3	2830.00	900.00	2020.00
$(\text{NH}_4)_2\text{SO}_4$	463.00	-	-
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	185.00	370.00	247.00
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	166.00	440.00	294.00
KH_2PO_4	400.00	170.00	-
KCl	-	-	1492.00
$\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$	-	-	312.00
Micro-nutrients			
KI	0.83	0.83	8.30
H_3BO_3	1.60	6.20	3.10
$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	4.40	15.60	11.20
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	1.50	8.60	7.20
$\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$	-	0.25	0.24
$\text{CuSO}_4 \cdot 6\text{H}_2\text{O}$	-	0.025	0.16
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	-	0.025	0.24
$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$	-	-	0.024
Iron source			
Na_2EDTA	37.30	37.30	37.30
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	27.80	27.80	27.80
Vitamins			
Myo-Inositol	100.00	100.00	100.00
Nicotinic Acid	1.00	5.00	-
Pyridoxine-HCl	1.00	0.50	0.05
Thiamine-HCl	1.00	0.50	0.50
Glycine	-	2.00	2.00
Amino Acids			
l-Glutamine	-	-	100.00
l-Arginine	-	-	100.00
l-Asparagine	-	-	100.00
Sucrose	30 g l^{-1}	30 g l^{-1}	45 g l^{-1}
Agar (Phytigel)	2.5 g l^{-1}	2.5 g l^{-1}	2.5 g l^{-1}
pH	5.7	5.7	6.0

soft-ware) indicates that callusing, somatic embryogenesis and rooting percentage was significantly high on modified Y₃ media among the three media compared. Results obtained through comparison of three basal media provide a foundation for further refinement of the *in vitro* regeneration protocol for *E. guineensis* Jacq. 'Dura', and could be useful in micro-propagation of *E. oleifera* and commercially cultivated oil palm, *E. guineensis* Jacq 'Tenera'.

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